Alcoholysis of *Vernonia anthelmintica* Seed Oil and Isolation of Methyl Epoxyoleate[1]

Abstract

The methyl esters of *Vernonia anthelmintica* seed oil were prepared without oxirane destruction by methanolysis using potassium methylate as catalyst. A single crystallization of the methyl esters from petroleum ether at −60C was found to be a convenient means of preparing large samples of methyl epoxyoleate of 93.6% purity in 92% of theory yield as based on oxirane titration. Further purification was achieved by recrystallization from petroleum ether at −40C to give a product at 97% purity in 54% of theory yield. Gas-liquid chromatography and thin layer chromatography along with associated techniques were employed to obtain the fatty acid composition of the crystallization fractions. Fifteen fatty acid methyl esters, including the methyl 12,13-epoxyoleate were identified.

Introduction

THERE IS CONSIDERABLE interest in seed oils containing epoxy-fatty acids owing to the potential value of these acids and their derivatives in the field of plastics, foams, resins, and surface coatings. *Vernonia anthelmintica* seed oil is receiving special attention (1,2) because of its high content (70%) of *cis*-12,13-epoxy-*cis*-9-octadecenoic acid (vernolic acid). The alkyl esters of epoxyoleic acid are known to be effective stabilizers for polyvinyl chloride plastics.

The methyl esters have been prepared from the acids of the Vernonia oil by reaction with diazomethane and the methyl epoxyoleate isolated by such techniques as adsorption and partition chromatography (3). This method for preparing methyl esters, however, is objectionable particularly for large quantities owing to the toxic nature of the reagent.

The objective of the present investigation was to determine whether alkyl esters could be made by alcoholysis of the Vernonia oil without destruction of the oxirane and to find a feasible method for isolating the esters of epoxyoleate in large batch operations. Alcoholysis at room temperatures or with short time reflux was effective in making complete conversion to methyl esters without loss of oxirane. Simple crystallizations permitted isolation of methyl vernolate in good yields. Analysis of the fractions of methyl esters of the oil gave more comprehensive information on all of the components.

Experimental

The oil of *V. anthelmintica* seed was obtained by extraction with warm petroleum ether. The seed had been autoclaved to inactivate enzymes before grinding (4).

Methanolysis. Previous work (5) had shown that under anhydrous conditions more than catalytic amounts of sodium or potassium methoxide in methanol accelerated the methanolysis and reduced the time and heating required. In a similar way a number of conditions were tried for methanolysis of small samples of Vernonia oil. The preparation of the methanol solutions of methoxide and the general procedure were the same as described previously (5). Several conditions gave complete conversion of 2 g samples to methyl esters: 50 ml of 0.02 N sodium methylate, 15 min reflux; 50 ml of 0.2 N potassium methylate, 2 hr at room temperature; and 50 ml of 0.02 N potassium methoxide overnight at room temperature.

Larger batches up to 200 g of the oil were converted equally effectively to methyl esters under the same conditions, except that the ratio of methoxide solution to sample was reduced to 13:1. The completeness of the methanolysis was determined by monitoring the reaction by thin-layer chromatography which gave a clear-cut separation of any unreacted glycerides from methyl esters. Gas-liquid chromatography of the isolated reaction product also gave confirmation of completeness of reaction as judged by comparing total area under the peaks of the chromatogram with that obtained from a similar mixture of known methyl esters when the same size samples were injected on the same column and under same conditions. Oxirane determinations on the oil and the ester product were made to detect whether any destruction of oxirane group had taken place.

Isolation of Methyl Vernolate. The crude reaction product of the methanolysis after acidification to about pH 3.5 with cold dilute sulfuric acid (dropwise with stirring) was promptly extracted with petroleum ether (bp 30–60C) and washed with distilled water until neutral. Care must be exercised in washing to prevent troublesome emulsions. The extract was dried over anhydrous sodium sulfate. The ratio of petroleum ether to product was adjusted to 12:1 v/w and the solution was subjected to crystallization at −60C and recrystallization of precipitate at −40C.

Gas-Liquid Chromatography. Gas-liquid chromatographic analyses on products and fractions were performed with apparatus described previously (6,7). The column was 8 ft × ¼ in. (ID = 0.180 in.) stainless steel tube packed with 42–60 mesh acid-base washed Chromosorb W coated with 20% ethylene glycol succinate polyester, and was operated at 204C. Owing to evidence of some alteration taking place in the epoxyoleate during GLC, for quantitative purposes it was found desirable to establish factors for the various components by making use of a known mixture of these components including also a known percentage of methyl erucate (known to be absent in the oil) as an internal standard. The area under the peak of each component was made relative to that of the standard. These factors when equated with the respective areas of the peaks and with the known percentage of the internal standard added to the esters of the oil permitted quantitative estimations to be made of each component.

Thin Layer Chromatography. Thin layer chromatoplates were prepared with Silica Gel G according to the procedure of Stahl (8). The solvent system de-

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TABLE I

Methanolysis of Vernonia Seed Oil [a]

Oil wt	KOME [b]	Time	Recovery		I.V.	Oxirane oxygen
g	*norm*	*hrs*	*g*	%		%
50.9	0.22	2	51.0	99.8	104.6	3.87
26.5	0.02	17	26.0	97.5	106.4	3.84
10.1	0.02	17	10.1	99.7	105.7	3.99

[a] Oxirane oxygen = 3.81, I.V. = 101.3, % FFA = 1.1.
[b] KOME solution to sample ratio, 13:1 (v/w).

scribed by Morris (9) was used to develop the chromatograms. Spraying developed plates with sulfuric acid and heating was employed to visualize the components on the chromatogram.

Oxirane. The percentage of oxirane oxygen was determined by the method of Durbetaki (10).

Results and Discussion

Table I shows data obtained from several typical experiments on methanolysis. The results indicate nearly quantitative recovery and no destruction of oxirane. The iodine numbers (Wijs) are somewhat variable. This has been observed regularly even on repeated analysis of the same oil or fraction containing epoxyoleic acid or ester. It is reasonable to expect that the reagent reacts to some extent with the oxirane ring. Thin-layer chromatography gave no evidence of any unreacted glycerides in the product.

The results of a small scale crystallization of the methyl esters of the oil are shown in Table II. The P-1 fraction contained 93.6% epoxyoleate and represented a theory yield of 92.3%. Recrystallization gave a product P-2 which contained 97.1% epoxyoleate, theory yield 54.2%. A portion of the latter on acetolysis gave dihydroxyoleic acid, mp 52–54C, specific rotation in ethanol, $[\alpha]_D^{27} = (-)$ 4.34, values in agreement with those reported by Bharucha (11). Thin layer chromatography of the precipitate and filtrate fractions gave the same R_f values for the epoxyoleate in each; hence, no indication of more than one position isomer (9).

The P_2, F_1 and F_2 fractions from the crystallizations and the original total methyl esters were subjected to analysis by GLC on polyester columns. Alteration of the methyl epoxyoleate during analysis was suspected for two reasons: chromatograms of substantially pure epoxyoleate always showed a secondary peak, following the principal peak, which was much too large to be explained on the basis of impurities (Fig. 1); injection of equal size samples of normal esters of fats and of mixtures of these esters containing substantial amounts of pure epoxyoleate always gave about 25% less total area under chromatogram peaks for the latter with the particular column and conditions employed.

More convincing evidence of alteration was obtained by collecting material from the middle portion of the principal peak (Fig. 2) and rechromatographing it on the same column. Again the secondary peak

TABLE II

Fractions Obtained by Crystallization of Methyl Esters of *V. anthelmintica* Seed Oil

Fraction	Wt	Oxirane oxygen	Epoxyoleate
	g	%	%
Original esters	24.36	3.84	74.6
P_1	17.96	4.84	93.6
F_1	5.82	0.94	18.3
P_2	10.13	5.00	97.1
F_2	7.82	4.65	90.3

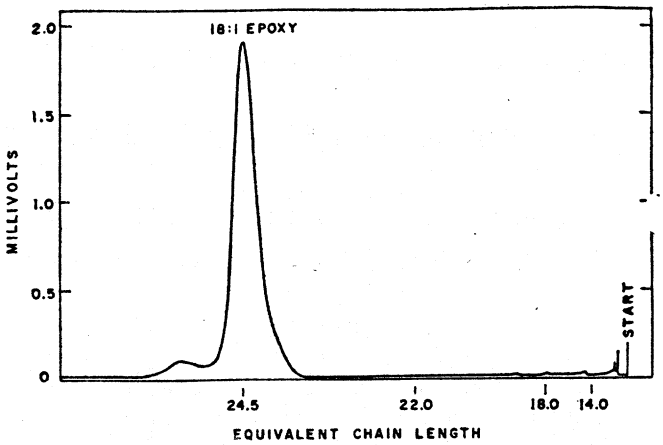

FIG. 1. Gas-lqiuid chromatogram of pure methyl epoxyoleate. Retention time 23 min, temp 204C, flow rate 75 ml/min.

appeared and had about the same area relative to that of the principal peak.

Further experiments were conducted in which a known percentage of methyl erucate was added to methyl esters of the oil, the crystallization fractions, and to known mixtures simulating the esters of Vernonia oil. Methyl erucate was chosen as internal standard because it is not a component of the oil and its retention time is about midway on the chromatogram well separated from other components. Calculations based on area relations compared to area and known percentage of standard showed clearly that only about 75% of the area expected from the epoxyoleate present appeared on the chromatogram. In other words, calculation of percentage epoxyoleate in sample gave values about 25% low based on the internal standard.

Examples of data so obtained are shown in Table III, along with data obtained by use of factors as well as internal standard as described in the experimental part of this paper. The unsaponifiable material was determined independently by the usual chemical method and was assumed to be the same in all calculated compositions shown. While the analysis using the internal standard and factors does not account for 100% of the sample, it does furnish reasonably good values for the individual components. The several pairs shown, such as 14:1 and 15:0, were not always separated on a given column. However, the use of several columns with different stationary phases particularly on the crystallization fractions provided evidence that both components were present. The presence of 18:3, 18:2 *c,t*, and

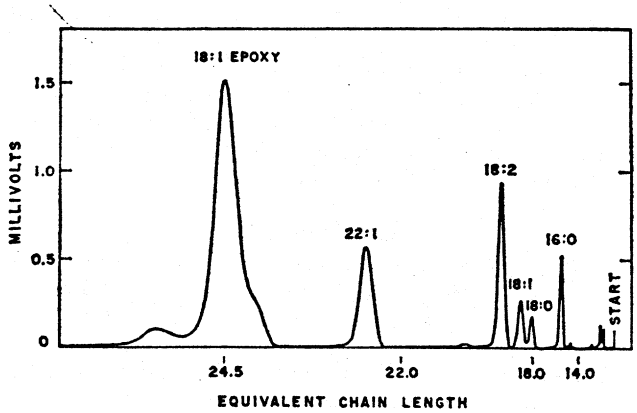

Fig. 2. Gas liquid chromatogram of methyl esters of *V. anthelmintica* seed oil to which methyl erucate was added (9.33%). Conditions same as for Fig. 1.

TABLE III

GLC Analysis of Vernonia Oil Methyl Esters Employing Internal Standard

Ester	Total esters [a]		Composite [b]	
	Without factors	With factors	Without factors	With factors
	%	%	%	%
<14:0	0.29	0.29	0.23	0.23
14:0	0.19	0.19	0.03	0.03
14:1 } 15:0 }	0.05	0.05	0.14	0.13
16:0	1.99	1.87	2.24	1.91
16:1 } 17:0 }	0.09	0.09	0.20	0.18
18:0	1.00	1.03	1.22	1.14
18:1	1.80	1.87	2.06	1.95
18:2	6.78	7.42	8.07	8.01
20:0	0.21	0.23	0.07	0.07
18:3 } Conj. 18:2 *c,t* }	0.33	0.36	0.41	0.41
Conj. 18:2 *t,t*	0.08	0.09	0.07	0.07
18:1 Epoxy	50.5	71.0	57.5	73.3
Unknown	.....	.....	1.36	1.24
Unsap.	7.76	7.76	7.76	7.76
Total	71.07	92.25	81.36	96.43

[a] GLC directly on total esters of oil.
[b] GLC on fractions from crystallization summated to original total esters.

18:2 *t,t* was more readily detected in the F_1 fraction owing to concentration of these by crystallization. A combination of gas-liquid chromatography, ultraviolet and infrared spectrophotometry was employed tablish their identity.

Further study is being made of the alteration of epoxyoleate during CLC analysis with the objective of finding more satisfactory column and stationary phase for analysis of oils containing epoxyoleic acid and possibly other oxygenated fatty acids. It is expected that the results will be reported soon.

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